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Progesterone receptor variant increases ovarian cancer risk in *BRCA1* and *BRCA2* mutation carriers who were never exposed to oral contraceptives

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Oral contraceptives have been shown to be protective against hereditary ovarian cancer. The variant progesterone receptor allele named *PROGINS* is characterized by an Alu insertion into intron G and two additional mutations in exons 4 and 5. The *PROGINS* allele codes for a progesterone receptor with increased stability and increased hormone-induced transcriptional activity. We studied the role of the *PROGINS* allele as a modifying gene in hereditary breast and ovarian cancer. The study included 195 *BRCA1* and *BRCA2* carriers with a prior diagnosis of ovarian cancer, 392 carriers with a diagnosis of breast cancer and 249 carriers with neither cancer. Fifty-eight women had both forms of cancer. Five hundred and ninety-five women had a BRCA1 mutation and 183 women had a *BRCA2* mutation. Overall, there was no association between disease status and the presence of the *PROGINS* allele. Information on oral contraception use was available for 663 of the 778 carriers of *BRCA1* or *BRCA2* mutations. Among the 449 subjects with a history of oral contraceptive use (74 cases and 365 controls), no modifying effect of *PROGINS* was observed [odds ratio (OR) 0.8; 95% confidence interval (CI) 0.5–1.3]. Among the 214 carriers with no past exposure to oral contraceptives, the presence of one or more *PROGINS* alleles was associated with an OR of 2.4 for ovarian cancer, compared to women without ovarian cancer and with no *PROGINS* allele ($P = 0.004$; 95% CI 1.4–4.3). The association was present after adjustment for ethnic group and for year of birth. Pharmacogenetics 11:635–638 © 2001 Lippincott Williams & Wilkins

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Introduction

The human progesterone receptor gene (*HPR*) belongs to the steroid-thyroid-retinoic acid receptor superfamily of transcription factors and is located on chromosome 11q22–23. HPR exists in two isoforms, HPR-A and HPR-B, transcribed from the *HPR* gene by alternative initiation. HPR-A represses oestrogen receptor gene activation and gene activation by HPR-B. Rowe *et al.* (1995) identified a 306-bp insertion of the PV/HS-1 Alu subfamily in intron 7 of the *HPR* gene. Kieback *et al.* (1998) demonstrated that the Alu insertion defines an allele, named *PROGINS*,

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which is in linkage disequilibrium with a silent mutation in exon 5 and a missense mutation in exon 4 causing an amino acid change in the hinge region of the receptor. *PROGINS* codes for a progesterone receptor with increased stability and increased hormone-induced transcriptional activity.

The role of *PROGINS* as a risk factor of ovarian cancer is unclear. Oral contraceptives contain progesterone, alone or in combination with oestrogen, and are protective against ovarian cancer (Risch, 1998; Rosenberg *et al.*, 1994). Oral contraceptives also prevent hereditary ovarian cancer in women who carry *BRCA1* or *BRCA2* mutations (Narod *et al.*, 1998).

Not all women who carry a *BRCA1* or *BRCA2* germline mutation develop breast or ovarian cancer, and both genetic and non-genetic modifiers of risk are implicated. The risk of developing ovarian cancer has been shown to be increased in association with rare (infrequent) alleles of the HRAS variable number tandem repeats polymorphism (Phelan *et al.*, 1996) and the risk of breast cancer is modified by the repeat length of the androgen receptor (Rebbeck *et al.*, 1999). It is possible that the protective effect of oral contraceptives in *BRCA1* and *BRCA2* carriers is modified by other genetic factors.

Therefore, we studied the role of the *PROGINS* allele in modifying breast and ovarian cancer risk of *BRCA1* and *BRCA2* mutation carriers, in particular with respect to the history of oral contraceptive use.

Materials and methods

Study population

We typed 778 female germline *BRCA1/2* mutation carriers for *PROGINS*. The carriers originated from 405 breast-ovarian cancer families identified in North America, and were collected in the course of several genetic and epidemiological studies. These women presented for genetic testing in the clinical centres affiliated with the collaborating institutions. There were 595 carriers of *BRCA1* mutations and 183 carriers of *BRCA2* mutations. One hundred and ninety-five of the 778 carriers had a history of ovarian cancer, 392 carriers had a history of breast cancer and 249 had no history of either cancer. Fifty-eight women had both ovarian and breast cancer. Three hundred and one of the carriers were Ashkenazi Jewish (38.7%), 90 were French-Canadian (11.6%), 366 were white, of other European origins (47.0%), and 21 were African-Americans (2.7%). The average age of diagnosis of ovarian cancer cases was 53.2 years and the average age of diagnosis of the breast cancer cases was 41.6 years. The average age of the women with neither form of cancer was 42.5 years.

Genotyping analysis of the PROGINS *allele*

The analysis of the polymerase chain reaction (PCR) fragments of exon 4, exon 5 and intron G for the *PROGINS* allele was carried out as described elsewhere (Kieback, 1998; Wang-Gohrke *et al.*, 2000). In brief, the analysis of the intron G insertion was based on the PCR amplification using the following primers: sense primer OL-334, 5′-GCC TCT AAA ATG AAA GGC AGA AAG C-3′, and anti-sense primer OL-335, 5′-GCG CGT ATT TTC TTG CTA AAT GTC TG-3′. The amplification in standard PCR buffer was for 30 cycles, each consisting of 1 min denaturing at 94 °C, 1 min of annealing at 60 °C and 1 min of extension at 72 °C. An initial denaturation step of 3 min at 94 °C and a final extension at 72 °C for 5 min were used. The A1 allele of *HPR* was defined as the absence of the insertion, according to previous publication (Rowe *et al.*, 1995). The A1 allele expectedly appeared as a 175 bp fragment, the A2 allele, *PROGINS*, as a 481 bp fragment.

Results

The *PROGINS* A2 allele was detected in 34.2% of the 778 *BRCA1/2* carriers, including 34.3% of the *BRCA1* carriers and 33.9% of the *BRCA2* carriers. The variant was present more often in the Jewish carriers (42.9%) than in French-Canadian (31.1%) or other white carriers (29.2%; $P < 0.001$) (Table 1). The frequency of the A2 allele was similar in carriers with breast cancer (33.9%), with ovarian cancer (37.4%), with both types of cancer (46.6%) and with neither cancer (34.9%). We observed no significant difference between the ages of diagnosis of ovarian or of breast cancer between women with and without the *PROGINS* allele. The average age at diagnosis for the 70 ovarian cancer cases with *PROGINS* was 54.8 years and for the 111 cases without *PROGINS* was 52.1 years ($P = 0.15$). The average age at diagnosis of breast cancer of the 131 cases with *PROGINS* was 42.2 years and the average age of the 246 cases without *PROGINS* was 41.3 years ($P = 0.70$). The data were analysed using a multivariate logistic regression model adjusting for year of birth, mutation and ethnicity. In these analyses, women with ovarian cancer were compared with women without ovarian cancer (women in either group may have had a past history of breast cancer). After adjustment, the odds ratio (OR) estimates associated with the *PROGINS* allele, were 1.11 for ovarian cancer [95% confidence interval (CI) 0.75–1.63] and 1.05 for breast cancer (95% CI 0.77–1.43). No associations were seen be-

Table 1. Prevalence of the *PROGINS* allele (%) according to ethnic background and disease status

Ethnic group	*Breast cancer*	*Ovarian cancer*	*Healthy*	*All*
Jewish ($n = 301$)	44.6	43.9	43.8	42.9
French-Canadian ($n = 90$)	35.6	27.3	28.9	31.1
Other Caucasian ($n = 366$)	27.0	29.1	32.8	29.2
African-American ($n = 21$)	16.7	0.0	0.0	9.5

tween the *PROGINS* allele and ovarian or breast cancer in either the *BRCA1* or the *BRCA2* subgroup.

Because of the importance of oral contraceptives (which contain progesterone) in ovarian cancer aetiology, we analysed separately the subgroups of women with and without a past history of oral contraceptive use (Table 2). Information on oral contraceptive use was available for 663 of the 778 study subjects, including 86% of women with ovarian cancer, 84% of women with breast cancer and 86% of women with neither form of cancer.

Table 2. Distribution of *PROGINS* genotypes in *BRCA1/2* mutation carriers with ovarian cancer (cases) and without ovarian cancer (controls) with respect to oral contraceptive use and non oral contraceptive use

	PROGINS *genotypes (%)*		
	A1/A1	A1/A2	A2/A2
Oral contraceptive users			
All carriers			
Cases ($n = 78$)	69.2	29.5	1.3
Controls ($n = 370$)	63.8	33.0	3.2
BRCA1 carriers			
Cases ($n = 65$)	64.6	33.9	1.5
Controls ($n = 282$)	63.1	33.7	3.2
BRCA2 carriers			
Cases ($n = 13$)	92.3	7.7	0
Controls ($n = 88$)	65.9	30.7	3.4
Non oral contraceptive users			
All carriers			
Cases ($n = 89$)	52.8	41.6	5.6
Controls ($n = 126$)	73.0	24.6	2.4
BRCA1 carriers			
Cases ($n = 62$)	50.0	43.6	6.4
Controls ($n = 89$)	75.3	21.4	3.4
BRCA2 carriers			
Cases ($n = 27$)	59.3	37.0	3.7
Controls ($n = 37$)	67.6	32.4	0

There were 449 women (68%) who had a past history of oral contraceptive use. Among the 214 subjects (89 cases and 125 controls) with no past exposure to oral contraceptives, the *PROGINS* allele was associated with an OR of 2.4 for ovarian cancer, compared to women without ovarian cancer ($P = 0.004$; 95% CI 1.4–4.3). Among the 449 subjects with a history of oral contraceptive use, no increased risk of ovarian cancer was observed (OR 0.8; 95% CI 0.5–1.3). The effect of *PROGINS* on ovarian cancer risk in non-users of oral contraceptives was significant only for *BRCA1* carriers (OR = 3.05; $P = 0.001$). In *BRCA2* carriers, the risk was modest and was non-significant (OR = 1.43; $P = 0.49$).

Among *BRCA1* carriers, the OR for women with one A2 allele (OR = 3.07) was similar to that for women with two A2 alleles (OR = 2.88); however, the number of women in the latter category was small ($n = 7$). Because of the observed ethnic differences in the allele frequencies of the A2 allele, we studied the association in *BRCA1* carriers who did not use the pill by ethnic groups. The OR was 1.94 ($P = 0.14$) for Jewish women; 2.00 ($P = 0.66$) for French-Canadian women and 5.1 ($P = 0.01$) for other white women. Combining these three estimates using the Mantel-Haenszel procedure, the ethnic group-adjusted OR was 2.5 ($P = 0.01$). Among women with exposure to oral contraceptives, the Mantel-Haenszel adjusted OR was 0.88 ($P = 0.69$). The interaction between oral contraceptive use and the presence of the *PROGINS* allele was statistically significant (Breslow-Day test for homogneity of ORs; $P = 0.009$).

The presence of the *PROGINS* allele did not vary by time. *PROGINS* was present in 32% of women born before 1940 and in 36% of women born in or after 1940 ($P = 0.35$). To adjust for year of birth, we stratified the sample by decade of birth. Among *BRCA1* carriers who did not use oral contraceptives, the OR for ovarian cancer was 7.5 for women born before 1920; 4.9 for women born between 1920 and

1940, and 0.8 for women born in or after 1940. Combining these three ORs using the Mantel-Haenszel technique, the age-adjusted OR was 2.2 ($P = 0.03$).

Discussion

The progesterone receptor is expressed in normal and neoplastic ovarian, breast and endometrial epithelial cells. In a recent population-based case–control study in Germany, we found *PROGINS* to be associated with reduced risk for sporadic (unselected) breast cancer before the age of 50 years (Wang-Gohrke *et al.*, 2000). Compared to wild-type A1/A1 homozygotes, the OR for A1/A2 heterozygotes was 0.82 (95% CI 0.62–1.08) and for A2/A2 homozygotes was 0.27 (95% CI 0.10–0.74). In this study of *BRCA1* and *BRCA2* mutation carriers, the *PROGINS* allele did not significantly modify breast cancer risk but did modify ovarian cancer risk, in particular with respect to the history of oral contraceptive use.

The women with ovarian cancer in our study were older, on average than the women without ovarian cancer. The average year of birth of the women with ovarian cancer was 1939, the average age of diagnosis was 53 years and the average age at genetic testing (study entry) was 58 years. These are prevalent cases and a mean of 5.1 years had elapsed between age of diagnosis of ovarian cancer and genetic testing. Among women without ovarian cancer, the average year of birth was 1949 and the average age at study entry was 48 years. Oral contraceptive use increased with calendar time, and was used by 32% of women who were born before 1940 and by 82% of women born in or after 1940. However, the main exposure of interest is the *PROGINS* allele, which did not vary by time.

The total number of patients in the present study is relatively large, although no single subgroup was particularly large and multiple comparisons were made. It is therefore possible that the effect seen here was due to chance, and it will be important to replicate these observations in a second population of carriers. Furthermore, we tested prevalent cases of ovarian cancer for the *BRCA1* and *BRCA2* status; an average of 5.1 years had elapsed from the time of diagnosis to genetic testing for women with ovarian cancer. If there is a relationship between the *PROGINS* allele and survival from ovarian cancer, then the results obtained from prevalent cases may not be representative for ovarian cancer in general.

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